Pak. J. Bot., 22(2): 197-203, 1990.

GREEN HOUSE EVALUATION OF SELECTED HERBICIDES FOR THEIR EFFECT ON SOYBEAN (*GLYCINE MAX* L.) GROWTH, NODULATION AND NITROGENASE ACTIVITY

C.A. OZAIR, L.J. MOSHIER* AND A.H. CHAUDHRY**

National Agricultural Research Centre,
Pakistan Agricultural Research Council, P.O. NARC,
Islamabad, Pakistan.

Abstract

Post-emergence herbicidies Acifluorfen (5-2 chlore-4-tri-fluoremethyl) Phenoxy-2-nitrobenzoic acid), Bentazon (3- (1-methylethyl)- (IH)-2, 1, 3-benzothiadiazin-4 (3H)-one 2,2-dioxide), chloramben (3-amino-2, 5-dischlorobenzoic acid), and 2, 4-DB (4- (2,4-dichlorophenoxy) butanoic-acid) @ 0.56, 1.12, 2.8 and 0.04 kg ai/ha, respectively and their two way combinations at similar rates were applied to soybean (*Glycine max*) at the V2-growth stage in 2 sets of green-house experiments. All treatments except Bentazon alone or when mixed with acifluorfen reduced the ethylene production (nitrogen fixation assay) 2 weeks after the herbicide spraying. In both studies, treatment effects were significant on either single or more parameters; shoot weight, root weight, nodule weight or nodule number. Herbicidal phytotoxicity on treated foliage showed an evidence of disruption at the cellular level which would temporarily reduce photosynthate production and the subsequent translocation. Within herbicides, only acifluorfen + bentazon and acifluorfe supressed the nitrogenase activity in green-house study. Variation associated with ARA (acetylene reduction assay) within the two sets of green-house studies may be due to different season of planting, method of sampling for ARA, light intensity and or temperature regimes. Data indicates that ethylene production on per plant basis rather than per nodule weight basis should be considered when evaluating effect of herbicides on nitrogen fixation.

Introduction

Soybean is an important crop for oil content and protein in grain. Nitrogen economy for this protein is balanced through its ability to fix atmospheric nitrogen via root nodules. Adverse effects of herbicides on nodulation have been reported for soybeans and other leguminous species. Kust & Struckmeyer (1971) found that treflan @ 0.4 to 0.7 kg ai/ha reduced nodulation in soybeans up to 8 weeks after spray. Carlyl & Thrope (1947) reported that 2, 4-D at a concentration of 0.5 ug/ml in a nutrient solution reduced nodulation of dry beans, alfalfa (*Medicago sativa*), grarden pea (*Pisum sativum*) and red clover (*Trifolium pratense*) grown in sand culture. A reduction in nodule number and size occured in garden peas treated at the 3-leaf stage with amitrol-T or dawpon (Johnen *et al.*, 1978). Mefluidide @ 0.28 and 0.42 kg ai/ha

* Department of Agronomy, Kansas State University, Manhattan, Kansas, U.S.A.

** Department of Biology, Quaid-e-Azam University, Islamabad.

reduced the soybean shoot weight and nodule weight indicating source-sink relationship with nodule size (Ozair & Moshier 1988 a). In another study Ozair & Moshier (1988 b) reported that aciflourfen reduced nitrogen fixation 2 weeks after the application.

Luckshmi-Kumari *et al.*, (1974) reported a reduction of nodule number due to reduced plant growth of alfalfa grown in nutrient solution containing amitrol @ 1 g/ml. Brock (1972) found that nodulation reduction was correlated with root growth reduction of several clover species (*Trifolium spp.*), in treflan treated soils. Nodulation reduction in alfalfa due to soil supplied balan or foliar applied moelon + butyric was associated with plant growth reduction (Peters & Banzbiba, 1979). A supply of photosynthate is essential for nitrogenase activity to be maintained within nodules (Wong & Evans, 1971). Gramaxon, which can cause rapid dessication of treated leaf (Anon., 1987), decreased nitrogenese activity in white clover (*Trifolium repense*) within 24 h after treatment (Rolston *et al.*, 1975).

On the contrary, Elfadi & Fahmy, 1958; Avrov, 1966; Ozair & Moshier, 1988a; Dunigan *et al.*, 1972) investigated that some herbicides did not reduce nodulation in treated plots. Herbicide applications before and/or after weed emergence is an important component in weed management programs. Herbicides are applied as post-emergence (after soybean and weed emerge) to control weed that escape herbicide applications at planting time. Effect of post-emergence herbicides on nodulation and nitrogenase activity for symbiotic N2-fixation in soybean plants is not known. The present report describes the results of a greenhouse study of the use of post-emergent herbicides viz., acifluorfen, bentazon, chloramben and 2, 4-DB on nodulation and nitrogen fixation capability in soybean.

Material and Methods

Soil sample was collected in February, 1982 from the Kansas State University Agronomy Farm from a site with Eudora silt loam *Fluventic hapludoll*, (coarse-silty, mixed mesic, sand 26%, silt 65%, clay 9%, pH 6.3, organic matter 1.4% (Anon., 1982). Soybean were grown on this site for the last 2 years. The soil collected at random from the upper 30 cm of the profile was sieved with 4-mm screen to remove plant debris and uniformly mixed. A soil - sand mixture (3:1) was filled in 946-ml paper pot to a depth of 12.5 cm. Soybean seeds cv. William treated with thiram fungicide (bis (dimethylthiocarbamoyl) disulfide) and 2 g/kg seed since it does not affect symbiotic nitrogen fixation (Curley & Burton 1975) were planted at 2.5 cm depth, 5 seeds per pot. Upon germination the seedlings were thinned to 3 per pot. Plants were watered as and when necessary and fertilized weekly with a half strength modified N-free Hoagland's nutrient solution. During first green house study no suplement light was used and the seasonal day length caused early flowering. However, short day stress was removed in second set of green-house study through supplying fluorescent light with a photoperiod of 15-h PPFD (Photosynthetic photon flux density) of 700 + 25μ mol m^{-2} $S^{-1}$to prevent early flowering (Thomas & Raper, 1983). The green house day/night aerial temperatures of 32:18 $\pm$ 2^{O}C was maintained. Pots were rotated on a green-house bench every 3-4 days to ensure uniform growth. Post-emergence herbi-

cides consisting of sodium salt of acifluorfen @ 0.6 kg ai/ha, sodium salt of bentazon @ 1.1 kg ai/ha, ammonium salt of chloramben @ 2.3 kg acid equivalent (ae)/ha, and dimethylamine salt of 2,4-DB @ 0.13 ae/ha were applied when plants were at V2 stage or when trifoliate leaf at second node was fully developed (*Fehr et al.,* 1971). Two way combinations of these herbicides were also applied at the same rate as and when applied alone. Nitrogenase activity for nitrogen fixation was determined by acetylene reduction to ethylene, indirect assay on a gas liquid chromatograph (Hardy *et al.*, 1969). Number of nodule, nodule fresh weight, shoot and root fresh weights were recorded. Treatments were replicated 4 times. Analysis of variance was performed by using completely randomized design except for ethylene produced per plant. This parameter was statistically analyzed using randomized complete block design since all plants from a replicate were harvested together and incubation time and temperature were kept constant for that replicate. Means were seperated by Duncan's Multiple Range test. Data presented for each set of green house study are means of two experiments.

Results and Discussion

None of the treatments significantly reduced soybean shoot weight or nodule frequency in the first set of green-house study. Root weight was significantly reduced by combination of acifluorfen with bentazon and chloranben and by bentazon mixed with chloramben. Nodule weight was reduced by acifluorfen + bentazon, bentazon + chloramben, bentazon + 2,4-DB. Except acifluorfen + bentazon, none of the treatment significantly reduced the ethylene production on per plant or per nodule weight basis (Table 1). In our second set of green-house study (Table 2), all treatments except bentazon alone, significantly reduced shoot weight of soybean. Root weight was also significantly reduced when plants were treated with mixture of bentazon + 2, 4-DB and bentazon + chloramben, all other herbicide treatments significantly decreased the nodule weight. Chloramben and acifluorfen with either bentazon or chloramben and mixture of bentazon + 2, 4-DB, signficantly reduced the nodule frequency. All treatments except bentazon alone showed a declining trend in ethylene production per plant, however, 5 of these treatments reduced ethylene production on unit nodule weight basis.

In green-house study-I, acifluorfen and bentazon when mixed together, significantly reduced the nitrogenase activity which indicates some sort of synergism as none of these herbicides significantly decreased the ethylene production when applied alone. On the contrary, green-house study reveals that except bentazon alone, all herbicide treatments significantly reduced the ethylene production over weedy check. However, variation in ethylene production within the herbicide treatments were non significant. Variations associated with ARA within the two sets of studies may be due to several factors such as; soybean were planted at different times of the year and therefore, grown under different light intensity regimes. No supplement lighting was used in first green-house study and only low intensity lighting was used in green-house study-II to extend photoperiod. Nitrogenase activity increased as the light intensity increased. Also, in our green-house study-I, roots were washed prior to ARA. Likely

Table 1. Effect of post-emergence herbicides on growth, nodulation and nitrogenese activity of soybean (green-house study-1 with no light supplement).

Herbicide	Rate	Shoot Weight	Root Weight	Nodule		Nitrogenase activity (Ethylene produced)	
				weight	frequency	per Plant	per nodule weight
	(Kg ai/ha)	(g/Plant)[a]		(no/Plant)[a]		(umoles/hr)[a]	(umoles/g/hr)[a]
Acifluorfen	0.6	3.92 a	4.03 abc	0.35 ab	35.6 b	4.2 ab	10.7 ab
Bentazon	1.1	3.63 abc	3.88 bc	0.32 ab	37.4 b	4.5 a	12.0 ab
Chloramben	2.8	3.60 abc	3.98 abc	0.34 ab	36.2 b	4.2 ab	12.6 a
2, 4-DB	0.04	3.75 abc	3.98 abc	0.36 abc	36.0 b	4.2 ab	11.7 ab
Acifluorfen + Bantazon	0.6+1.1	3.84 ab	3.50 c	0.32 b	36.4 b	2.8 b	7.9 b
Acifluorfen + Chloramben	0.6+2.8	3.57 abc	3.75 c	0.35 ab	31.4 b	4.9 a	13.2 a
Acifluorfen + 2,4-DB	0.6+0.04	3.37 c	4.03 abc	0.36 ab	31.9 b	4.3 ab	10.8 ab
Bantazon + Chloramben	1.1+2.8	3.63 abc	3.70 c	0.32 b	32.2 b	4.1 ab	12.7 a
Bantazon + 2, 4-DB	1.1+0.04	3.50 bc	4.38 ab	0.32 b	37.3 b	5.3 a	13.8 a
Check	None	3.6 abc	5.56 a	0.37 a	44.4 a	7.7 a	14.9 a

Means within columns followed by common letters are not significantly different according to Duncan's multiple range test at 5% level.

Amiben	chloramben	3-amino-2,5-dichlorobenzoic acid
Basagran	bentazon	3-(1-methylethyl)-1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Blazer	acifluorfen	5-(2-chloro-4(trifluoromethyl) phenoxy-2-nitrobenzoic acid
Butyrac	2,4-DB	4-(2,4-dichlorophenoxy) butanoic acid

Table 2. Effect of post-emergence herbicides on growth, nodulation and nitrogenase activity of soybean (green-house study-2 under fluorescent light

Herbicide	Rate	Shoot Weight	Root Weight	Nodule		Nitrogenase activity (Ethylene produced)	
				weight	frequency	per Plant	per nodule weight
	(Kg ai/ha)	(g/Plant)[a]			(no/Plant)[a]	(umoles/hr)[a]	(umoles/g/hr)[a]
Acifluorfen	0.6	5.90 c	2.68 ab	0.75 b	35.1 ab	7.4 cd	10.0 c
Bentazon	1.1	6.83 ab	2.83 ab	0.87 a	39.5 ab	11.4 ab	12.8 abc
Chloramben	2.8	6.51 bc	2.84 ab	0.73 bc	35.6 ab	7.9 cd	10.5 bc
2,4-DB	0.04	6.40 c	2.88 ab	0.78 ab	35.9 ab	10.1 bcd	13.1 abc
Acifluorfen + Bentazon	0.6+1.1	6.19 bc	2.66 ab	0.75 b	31.6 b	9.4 b cd	12.1 abc
Acifluorfen + Chloramben	0.6+2.8	5.81 c	2.89 ab	0.71 bc	31.7 b	7.8 cd	11.0 bc
Acifluorfen 2,4-DB	0.6+0.04	6.08 bc	2.57 ab	0.73 bc	35.2 ab	9.9 bcd	14.1 abc
Bentazon + Chloramben	1.1+2.8	6.27 bc	2.68 ab	0.81 ab	36.8 ab	8.6 bcd	10.7 bc
Bentazon + 2,4-DB	1.1+0.04	6.36 bc	2.48 b	0.72 bc	31.6 b	10.5 bc	14.4 ab
Check	None	7.40 a	3.06 a	0.89 a	41.4 a	13.5 a	15.9 a

Means within columns followed by common letters are not significantly different according to Duncan's multiple range test at 5% level.

difussion of oxygen during washing process would have caused inactivation of nitrogenase enzyme which is very sensitive to oxygen (Dobereiner *et al*, 1972, Evans & Barber, 1977).

Nodule weight and nitrogenase activity are influenced by stage of growth (Creaves *et al*., 1978). Nitrogenase activity is also affected by time of the day (Hardy & Havelka, 1976). Thus effect of herbicides on these parameters may have varied between experiments.

All treatments except bentazon and 24-DB alone and the mixture of bentazon + chloramben reduced nodule weight in the green-house study-11. Therefore, magnitude of ethylene production calculated on pre-nodule weight basis were much closer to the values for untreated plants than when calculated on per plant weight basis. Our

data indicates that ethylene production on per plant basis rather than per nodule weight basis should be considered when evaluating effect of herbicides on nitrogen fixation.

Translocation of the said herbicides and their metabolities are essentialy immobile in leguminous plants as reported for acifluorfen (Ritter & Coble, 1981), bentazon (Manhoney & Penner, 1975), chloramben (Ozair *et al.*, 1987) and 2, 4-DB (Kobertson & Kurkwood, 1970). It is therefore, unlikely that sufficient amount of these herbicides or their metabolites would translocate into the nodules or treated soybean plants and directly interfere with the nitrogenase. Reduction in nitrogenase activity would appear to be a secondary rather than primary response to these herbicide treatments. Therefore, injury from application of these herbicides would occur predominantly in treated tissue. The injury that was observed in treated tissue was evidence of disruption at the celluler level which would temporarily arrest shoot growth since effeciency of photosynthate production and translocation would be reduced.

Acknowledgement

Valuable assistance of Dr. P. Wong, Professor of Plant Physiology at Kansas State University, Manhattan, U.S.A., is gratefully acknowledged.

References

Anonymous. 1982. *Soil classification Bulletin*. Crops and Soils-5 Cooperative Extension Services, Kansas State University, Manhattan, KS. 66506. U.S.A.

Anonymous. 1983. *Weed Science Society of America*. Herbicides Hand-book. 5th Edn. Weed Sci. Am. Champaign, IL. pp. 362-364.

Avrov, O.E. 1966. Effect of herbicides on nodule bacteria and nodule formation in legumes. *Dokl. Vses. Akad. Sel. khy 2 Nauk* 3: 16-19. Abstract in Soils-Fert., 29:454.

Brock, J.L. 1972. Effect of the herbicides trifluralin and carbetamide on nodulation and growth of legume seedlings. *Weed Res.*, 12: 150-154.

Carlyl, R.E. and J.D. Thorpe. 1947. Some effects of ammonium and sodium salts of 2, 4-dichlorophenoxyacetic acid on legumes and the *Rhizobium* bacteria. *J. Amer. Soc. Agron.*, 39: 929.

Curley, R. L. and J.C. Burton. 1975. Compatiability of *Rhizobium japonicum* with chemical seed protectants. *Agron. J.*, 67: 807-808.

Craft, A.S. 1966. Comparative movement of labelled tracers in bean and barley. Isotopes in Weed Research. *Proc. Symp. I.A.E.A. Vienna*: 212-214.

Dobereiner, J., J.M. Day and P.J. Dart. 1972. Rhizosphere associations between grasses and nitrogen fixing bacteria. Effect of oxygen on nitrogenase activity in rhizosphere of *Paspalum notatum*. *Soil. Biol. Biochem.*, 5: 157-159.

Dunigan, E.P., J.D. Frey, L.D. Allen Jr. and A. Memahon. 1972. Herbicidal effects on the nodulation of *Glycine max* (L.) Merr., *Agron. J.*, 64: 806-808.

Elfadi, M.A. and M. Fahmy. 1958. Effect of sodium 2,4-D and MCPA on root nodulation of legumes and soil micro-organisms. *The Agric. Res. Rev.*, 36: 333-338.

Evans, H.J. and L.E. Barber. 1977. Biological nitrogen fixation for food and fiber producation. *Science*, 197: 332-339.

Fehr, W.R., C.F. Caviness, D.T. Burmood and J.S. Pennington 1971. Stage of development descriptions for soybeans (*Glycine max* (L.) Merr. *Crop Sci.,* 11: 929-93.

Greaves, M.P., L.A. Lockhat, and W.G. Richard. 1978. Measurement of herbicide effects on nitrogen fixation by legumes. Proc. 1978. British Crop Protection Conference: 581-584.

Hardy, R.W. and V.D. Havelka. 1976. Photosynthate as a major factor limiting nitrogen fixation by field grown legumes with emphasis on soybeans. pp. 421-437 *In*: *Symbiotic N2-fixation in plants. (Ed.) P.S. Nutman. Cambridge Univ. Press, Cambridge.*

Hardy, R.W., R.D. Holsten., E.K. Jackson and R.C. Burns. 1969. The acetylene-ethylene assay for nitrogen fixation. *Laboratory and field evaluation plant physiol.,* 43: 1185-1207.

Johnen, B.G., E.A. Drew and D.L. Castle. 1978. Studies on the effects of pesticides on symbiotic nitrogen fixation. *Proc. IX Int. Congr. Plant Pathology.*

Kust, C.A., and B.F. Struckmeyer. 1971. Effect of trifluralin on growth, nodulation and anatomy of soybeans. *Weed Sci.,* 19: 147-152.

Lukshmi Kumari, M., A. Biswas., K. Vigayalakshimi., H.S. Naryauna and N.S.S. Rao. 1974. Effect of certain water soluble herbicides on legume *Rhizobium* aymbiosis. Proc. *Indian Nat. Sci. Acad.*, 40: 529-539.

Manhoney, M.D. and P. Donland. 1975. Bentazon translocation and metabolism in soybean and navy bean. *Weed Sci.,* 23: 265-271.

Ozair, C.A. and L.J. Moshier. 1988a. Effect of soil-incorporated trifluralin and selected post-emergent herbicides on growth, nodulation and nitrogen fixation of soybean. *Pak. J. Agr. Res.,* 9: 316-320.

Ozair, C.A. and L.J. Moshier. 1988b. Effect of post-emergence herbicides on nodulation and nitrogen fixation in soybeans (*Glycine max*). *Applied Agr. Res.,* 3: 214-219.

Ozair, C.A., L.J. Moshier and G.M. Werner 1987. Absorption, translocation and metabolism of foliage applied chloramben in velvetleaf (*Abutilon theophrasti*) and soybean (*Glycine max*). *Weed Sci.,* 35: 757-762.

Peters, E.J. and M. Benzbiba. 1979. Effect of herbicides on nitrogen fixation of alfalfa (*Medicago sativa*) and red clover *(Trifolium pratense*). *Weed Sci.,* 27: 18-21.

Rolston, W.P., A.C.P. Chu and R.P. Fillery. 1975. Effect of paraquat on the nitrogen fixing activity of white clover (*Trifolium repense* L.). *N.Z.J. Ag. Res.,* 19: 47-49.

Ritter, R. L. and H.D. Coble. 1981. Penetration, translocation, and metabolism of acifluorfen in soybean (*Glycine max*), common ragweed (*Ambrosia artemisifolia*) and common coculebur (*Xanthium pensylvanicum*). *Weed Sci.,* 29: 474-484.

Thomas, J.F. and C.D. Raper Jr. 1983. Photoperiod and temperature regulation of floral initiation and anthesis in soybean. *Ann. Bot.,* 51: 481-489.

Wong, P.P. and H.T. Evans. 1971. Poly-B-hydroxy butrate utilization by soybean (*Glycine max*) nodule and assesment of its role in maintenance of nitrogenase activity. *Plant Physiol.,* 47: 750-755.

(Received for publication 20 March 1989)